Review | Vol. 10, No. 6 | 1 Jun 2019 | BIOMEDICAL OPTICS EXPRESS 2883
Biomedical Optics EXPRESS

Laser-assisted optoporation of cells and tissues – a mini-review

HERBERT SCHNECKENBURGER*

Aalen University, Institute of Applied Research, Beethovenstraße. 1, D-73430 Aalen, Germany
**herbert.schneckenburger@hs-aalen.de*

Abstract: Laser microbeam techniques are presented, which permit the introduction of molecules or small particles into living cells. Possible mechanisms – including photochemical, photothermal and opto-mechanical interactions (ablations) – are induced by continuous wave (cw) or pulsed lasers of different wavelength, power, and mode of operation. Laser-assisted optoporation permits the uptake of fluorescent dyes as well as DNA plasmids for cell transfection, and, in addition to its broad application to cultivated cells, may have some clinical potential.

1. Introduction

Shortly after the invention of the laser by Theodore Maiman in 1960 scientists applied its light focusing properties to biological systems, e.g. living cells and tissues [1]. Numerous applications of the laser microbeam technique – including selective illumination of cell compartments or organelles, structural and functional studies, optical trapping and manipulation or microdissection – have been reported and summarized, e.g. in [2–4]. While laser microbeams are often used for measurement or imaging of biological parameters as well as for trapping or moving of cells in an optical tweezer system, the present mini-review is focused on micromanipulation or microdissection techniques for introducing molecules or small particles into a cell. Such techniques offer an alternative to injection via micro-needles, as summarized in [5,6], and appear promising where the mechanisms involved are reversible and where cell viability can be maintained.

Often molecules or small vesicles are taken up by cells due to passive diffusion through the cell membrane or by endocytosis. Another possibility is active pumping of metabolites through membrane proteins. If, however, none these mechanisms work, laser microscopy techniques may support the uptake, e.g. of membrane impermeable fluorescent dyes or DNA plasmids. In the latter case genes of a foreign organism can be introduced into a native genome in order to modify the functional or fluorescent properties of a cell. This process is called transfection and represents a main application of laser-assisted optoporation. The mechanisms involved include photochemical, photothermal and opto-mechanical interactions (ablations), as described in the following section.

2. Mechanisms

Interaction of laser radiation with cells or tissues may vary depending on the wavelength of irradiation as well as on the light exposure. Fairly low light doses, around 100 J/cm^2 in the visible or near ultraviolet spectral range, can induce *photochemical* interactions [7]. These doses increase considerably in the near infrared range, and if instead of whole cells only small areas around 1 µm^2 are irradiated, light doses up to some hundred MJ/cm^2 can be applied without photochemical cell damage [8]. Photochemical interactions are related to absorption by endogenous molecules with photosensitizing properties, e.g. nicotinamide adenine dinucleotide (NADH), flavins or porphyrins at wavelengths below 650 nm. In the near infrared range, photochemical damage is lower, however two-photon absorption by endogenous molecules and (one-photon) absorption by water should be considered as dose-

#363181 https://doi.org/10.1364/BOE.10.002883
Journal © 2019 Received 25 Mar 2019; revised 9 May 2019; accepted 10 May 2019; published 17 May 2019

Biomedical Optics EXPRESS

limiting factors (measured according to the reduction in cell cloning efficiency [9,10]). Often photochemical reactions are irreversible and used for photochemical internalization or photodynamic therapy, e.g. in the treatment of cancer. Only in individual cases can they also be used for optoporation without risk of lethal damage [11,12], e.g. in the case of gene transfection or gene therapy.

Often laser-assisted optoporation is associated with *photothermal* interactions, which in a certain range of light dosage have proven to be reversible. This is demonstrated in Fig. 1, where small spots of 1.0 µm diameter were irradiated with a cw argon ion laser at 488 nm and a light dose of 2.5 MJ/cm^2 (applied for 2.5 s). After irradiation, tiny black spots – often surrounded by interference rings (marked by an arrow) – could be seen, however these disappeared within about five minutes [13,14]. At higher light exposure (≥ 5 MJ/cm^2), permanent changes of morphology were observed, and this was concomitant with lethal damages, as evidenced by a colony formation assay [14].

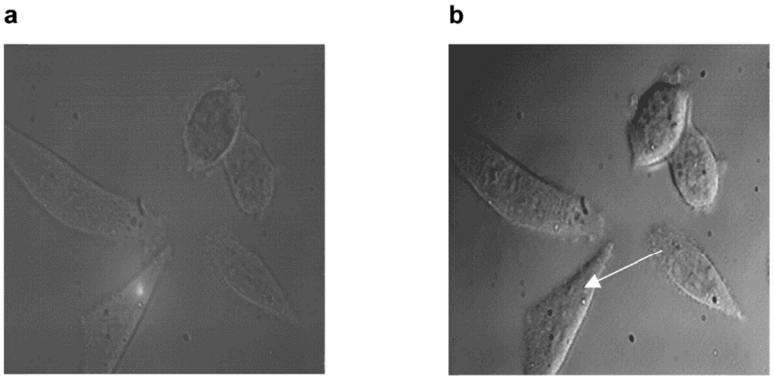

Fig. 1. CHO cells (a) during laser irradiation (phase contrast) and (b) after irradiation (interference contrast) (488nm; 1 MW/cm^2; 2.5 s; image size: 100 × 100 µm^2). The arrow marks the irradiated spot. Cells were kept in F-10 HAM medium with an increased amount of light absorbing phenol red (40 µM). Reproduced from Ref. 14 with modifications.

An explanation for the transient changes observed in Fig. 1 was given in [14]: by using a temperature-sensitive fluorescent membrane marker, an increase in temperature from about 35°C to 41°C was observed. In this temperature range, a phase transition of membrane lipids from a rather rigid gel phase to a more fluid liquid crystalline phase occurs [15], which may explain why certain molecules are taken up by cells more easily. After addition of a Green Fluorescent Protein (GFP) encoding plasmid DNA to the cultivation medium, the transfection rate of Chinese Hamster Ovary (CHO) cells (visualized by their green fluorescence) was increased from about 5% to 15–30% due to laser-assisted optoporation [14]. Upon application of cw lasers cell transfection was further observed in [11,13,16], and upon use of a laser scanning microscope specific cell types could be selected from a larger cell collective for transfection [16]. In further investigations, composite nanoshells [17] or magnetic carbon nanoparticles [18] were used as absorbers to create an appropriate heat profile for optoporation.

While cw lasers induced mainly thermal interactions, short-pulse (picosecond or femtosecond) lasers applied only a few years later induced local *ablation* and transient opening of cell membranes, so that exogenous material and even macromolecules could be introduced into living cells without photo-destructive effects. High repetition pulses from a mode-locked laser [19] or single near-infrared laser pulses were applied for this purpose [20]. In [21] cell viability and transfection efficiency were determined after application of millions of low-energy pulses as well as two higher-energy pulses. The data showed that pore size was the key factor in cell viability, independent of the laser irradiation regime. An important step towards automation was the introduction of a continuous flow system, offering the prospect

Biomedical Optics EXPRESS

of high-throughput optoporation [22]. Reducing the laser pulse duration down to 25 femtoseconds increased the optoporation rate, thus confirming the importance of multiphoton effects for this mechanism [23]. Use of femtosecond lasers in combination with plasmonic gold nanoparticles further enhanced the efficiency of optoporation, since, due to an amplified localized electromagnetic field, the membrane permeability of human melanoma cells increased. Thus, a very high perforation rate of 70%, a transfection efficiency three times higher than for conventional lipofection and very low toxicity (<1%) were obtained [24].

When using a nanosecond Nd:YAG laser instead of a femtosecond laser, 25–30% of the cells were perforated at low light doses of 50 mJ/cm^2 at 532 nm, or 1 J/cm^2 at 1064 nm [25]. Targeting of diseased cells with functionalized gold nanoparticles contributed to an even more selective treatment of these cells [26]. It should be emphasized that upon application of femtosecond laser pulses, in addition to ablation, plasmonic photoionization [27] as well as thermal effects have also been described. A comparison of high repetition infrared laser pulses (1.55 µm) with cw laser irradiation of the same wavelength and average power showed that in the first case a temperature gradient was generated which was more favorable to permeabilization of cell membranes [28].

3. Applications

For staining cells or organelles with fluorescent dyes, their passive diffusion through the cell membrane or uptake via specific carrier systems, e.g. micelles or liposomes, is commonly used. However, cell membranes are impermeable to certain actin-staining dyes, e.g. rhodamine phalloidin [29]. In this case, laser-assisted optoporation supports the cellular uptake of these dyes, permitting visualization of the cytoskeleton. Laser-assisted cell transfection – as an alternative process to lipofection, electroporation or viral transfection (as described e.g. in [30,31]) – probably represents the broadest field of application of laser optoporation. Use of liposomes as a carrier system for DNA plasmids is reported to support their delivery to the cell nucleus and increase the transfection rate [32]. It should be mentioned that laser-assisted optoporation has often been used in combination with a laser tweezer system, where cells or particles can be trapped and moved into the focus of a (second) laser beam [3], for precise localization or interaction with microparticles [33–35].

A first step towards *clinical application* is represented by the delivery of impermeable substances into retinal explants after ultrafast laser microbeam-assisted injection [36]. Further work by the same group includes optoporation of impermeable molecules to functional cortical neurons, leading to visualization of the actin network in the growth cone, as well as delivery of impermeable molecules into targeted retinal cells in a rat's eye. This may improve visualization of the structure and function of the retina [37]. In vivo optoporation of retinal ganglion cells (RGCs) targeted with functionalized gold nanoparticles was used to label these cells specifically with fluorescent conjugates. This provides a novel approach to selectively targeting retinal cells in diseased regions while sparing neighboring healthy areas [38]. Furthermore, local ablation and injury to individual cells by a laser microbeam was used to study the calcium metabolism around epithelial wounds. Calcium influx was measured in two steps: first to the damaged cell and about 45 s later to adjacent cells, which may also have been damaged by a cavitation bubble. This demonstrated that multiple mechanisms may accompany the process of optoporation [39].

A laser microdissection and pressure catapulting technique (LMPC) has been developed [40] for the characterization of single cells and their diverse biomolecules. With LMPC, the force of focused laser light is utilized to excise selected cells or large tissue areas from object slides down to individual single cells and subcellular components like organelles or chromosomes. After microdissection, the sample is directly catapulted into an appropriate recipient vial. As this process works entirely without mechanical contact, it enables pure sample retrieval from a morphologically defined origin. LMPC has been successfully applied to isolate and catapult cells from histological tissue sections, from forensic material as well as

Biomedical Optics EXPRESS

from tough plant matter. Combining LMPC with microinjection to inject drugs or genetic material into individual cells and capture them for molecular analysis holds great promise for the provision of efficient customized patient medication. The principles and further applications of this technique have been described and summarized more recently in [41,42].

4. Conclusion

The diverse mechanisms and applications of laser-assisted optoporation, as depicted graphically in Fig. 2 are still not fully understood. They can be induced by one- or multi-photon absorption of focused laser light and are sensitive to the wavelength, power and mode of operation (cw or pulses of variable duration and repetition rate). In all cases, the impact of the light dose should be examined carefully, so that transient perforation is induced and lethal damage avoided. Usually laser optoporation of a larger cell collective occurs sequentially, e.g. in a laser scanning microscope or a microfluidic system. However, simultaneous optoporation by a two- or even three-dimensional pattern of laser spots, as reported e.g. for holographic laser tweezers [43] or for a holographic photolysis system [44], might increase the speed of cellular uptake of metabolites or cell transfection in the future.

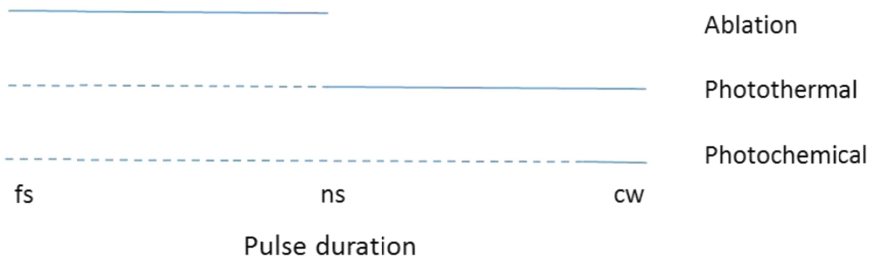

Fig. 2. Mechanisms causing laser-assisted optoporation (full lines: predominant range; broken lines: possible range).

Disclosures

The authors declare that there are no conflicts of interest related to this article.

References

1. S. Fine, T. H. Maiman, E. Klein, and R. E. Scott, "Biological effects of high peak power radiation," Life Sci. **3**(3), 209–222 (1964).
2. M. W. Berns, "A history of laser scissors (microbeams)," Methods Cell Biol. **82**, 1–58 (2007).
3. A. Ashkin, "Optical trapping and manipulation of neutral particles using lasers," Proc. Natl. Acad. Sci. U.S.A. **94**(10), 4853–4860 (1997).
4. K. O. Greulich, "Micromanipulation by light in biology and medicine: the laser microbeam and optical tweezers," Birkhäuser, Basel-Boston-Berlin, 1999.
5. D. V. McAllister, M. G. Allen, and M. R. Prausnitz, "Microfabricated microneedles for gene and drug delivery," Annu. Rev. Biomed. Eng. **2**(1), 289–313 (2000).
6. J. McCaffrey, R. F. Donnelly, and H. O. McCarthy, "Microneedles: an innovative platform for gene delivery," Drug Deliv. Transl. Res. **5**(4), 424–437 (2015).
7. H. Schneckenburger, P. Weber, M. Wagner, S. Schickinger, V. Richter, T. Bruns, W. S. L. Strauss, and R. Wittig, "Light exposure and cell viability in fluorescence microscopy," J. Microsc. **245**(3), 311–318 (2012).
8. T. Bruns, L. Becsi, M. Talkenberg, M. Wagner, P. Weber, U. Mescheder, and H. Schneckenburger, "Microfluidic system for single cell sorting with optical tweezers," in: Laser Applications in Life Sciences (M. Kinnunen, R. Myllylä, eds.), Proc. SPIE, **Vol.** 7376, 73760M (2010).
9. H. Liang, K. T. Vu, P. Krishnan, T. C. Trang, D. Shin, S. Kimel, and M. W. Berns, "Wavelength dependence of cell cloning efficiency after optical trapping," Biophys. J. **70**(3), 1529–1533 (1996).

Biomedical Optics EXPRESS

10. H. Schneckenburger, A. Hendinger, R. Sailer, M. H. Gschwend, W. S. L. Strauss, M. Bauer, and K. Schütze, "Cell viability in optical tweezers: high power red laser diode versus Nd:YAG laser," J. Biomed. Opt. **5**(1), 40–44 (2000).
11. C. P. Yao, Z. X. Zhang, R. Rahmanzadeh, and G. Huettmann, "Laser-based gene transfection and gene therapy," IEEE Trans. Nanobioscience **7**(2), 111–119 (2008).
12. A. Bucharskaya, G. Maslyakova, G. Terentyuk, A. Yakunin, Y. Avetisyan, O. Bibikova, E. Tuchina, B. Khlebtsov, N. Khlebtsov, and V. Tuchin, "Towards effective photothermal/photodynamic treatment using plasmonic gold particles," Int. J. Mol. Sci. **17**(8), E1295 (2016).
13. G. Palumbo, M. Caruso, E. Crescenzi, M. F. Tecce, G. Roberti, and A. Colasanti, "Targeted gene transfer in eucaryotic cells by dye-assisted laser optoporation," J. Photochem. Photobiol. B **36**(1), 41–46 (1996).
14. H. Schneckenburger, A. Hendinger, R. Sailer, W. S. L. Strauss, and M. Schmitt, "Laser-assisted optoporation of single cells," J. Biomed. Opt. **7**(3), 410–416 (2002).
15. T. Parasassi, G. De Stasio, G. Ravagnan, R. M. Rusch, and E. Gratton, "Quantitation of lipid phases in phospholipid vesicles by the generalized polarization of Laurdan fluorescence," Biophys. J. **60**(1), 179–189 (1991).
16. A. V. Nikolskaya, V. P. Nikolski, and I. R. Efimov, "Gene printer: laser-scanning targeted transfection of cultured cardiac neonatal rat cells," Cell Commun. Adhes. **13**(4), 217–222 (2006).
17. Y. A. Avetisyan, A. N. Yakunin, and V. V. Tuchin, "Novel thermal effect at nanoshell heating by pulsed laser irradiation: hoop-shaped hot zone formation," J. Biophotonics **5**(10), 734–744 (2012).
18. L. Gu, A. R. Koymen, and S. K. Mohanty, "Crystalline magnetic carbon nanoparticle assisted photothermal delivery into cells using CW near-infrared **laser** beam," Sci. Rep. **4**(1), 5106 (2014).
19. F. Stracke, I. Rieman, and K. König, "Optical nanoinjection of macromolecules into vital cells," J. Photochem. Photobiol. B **81**(3), 136–142 (2005).
20. H. Schinkel, P. Jacobs, S. Schillberg, and M. Wehner, "Infrared picosecond laser for perforation of single plant cells," Biotechnol. Bioeng. **99**(1), 244–248 (2008).
21. A. A. Davis, M. J. Farrar, N. Nishimura, M. M. Jin, and C. B. Schaffer, "Optoporation and genetic manipulation of cells using femtosecond laser pulses," Biophys. J. **105**(4), 862–871 (2013).
22. H. G. Breunig, A. Uchugonova, A. Batista, and K. König, "High-throughput continuous flow femtosecond laser-assisted cell optoporation and transfection," Microsc. Res. Tech. **77**(12), 974–979 (2014).
23. H. G. Breunig, A. Batista, A. Uchugonova, and K. König, "Cell optoporation with a sub-15 fs and a 250-fs laser," J. Biomed. Opt. **21**(6), 60501 (2016).
24. J. Baumgart, L. Humbert, É. Boulais, R. Lachaine, J. J. Lebrun, and M. Meunier, "Off-resonance plasmonic enhanced femtosecond laser optoporation and transfection of cancer cells," Biomaterials **33**(7), 2345–2350 (2012).
25. B. St-Louis Lalonde, E. Boulais, J. J. Lebrun, and M. Meunier, "Visible and near infrared resonance plasmonic enhanced nanosecond laser optoporation of cancer cells," Biomed. Opt. Express **4**(4), 490–499 (2013).
26. E. Bergeron, C. Boutopoulos, R. Martel, A. Torres, C. Rodriguez, J. Niskanen, J. J. Lebrun, F. M. Winnik, P. Sapieha, and M. Meunier, "Cell-specific optoporation with near-infrared ultrafast laser and functionalized gold nanoparticles," Nanoscale **7**(42), 17836–17847 (2015).
27. L. Minai, A. Zeidan, D. Yeheskely-Hayon, S. Yudovich, I. Kviatkovsky, and D. Yelin, "Experimental Proof for the Role of Nonlinear Photoionization in Plasmonic Phototherapy," Nano Lett. **16**(7), 4601–4607 (2016).
28. A. L. Garner, V. B. Neculaes, M. Deminsky, D. V. Dylov, C. Joo, E. R. Loghin, S. Yazdanfar, and K. R. Conway, "Plasma membrane temperature gradients and multiple cell permeabilization induced by low peak power density femtosecond lasers," Biochem. Biophys. Rep. **5**, 168–174 (2015).
29. K. Dhakal, B. Black, and S. Mohanty, "Introduction of impermeable actin-staining molecules to mammalian cells by optoporation," Sci. Rep. **4**(1), 6553 (2014).
30. T. Muramatsu, A. Nakamura, and H. M. Park, "In vivo electroporation: a powerful and convenient means of nonviral gene transfer to tissues of living animals," Int. J. Mol. Med. **1**(1), 55–62 (1998).
31. L. Kaestner, A. Scholz, and P. Lipp, "Conceptual and technical aspects of transfection and gene delivery," Bioorg. Med. Chem. Lett. **25**(6), 1171–1176 (2015).
32. A. Ishii, Y. Hiruta, D. Heinemann, A. Heisterkamp, H. Kanazawa, and M. Terakawa, "Intracellular localization and delivery of plasmid DNA by biodegradable microsphere-mediated femtosecond laser optoporation," J. Biophotonics **10**(12), 1723–1731 (2017).
33. M. Waleed, S. U. Hwang, J. D. Kim, I. Shabbir, S. M. Shin, and Y. G. Lee, "Single-cell optoporation and transfection using femtosecond laser and optical tweezers," Biomed. Opt. Express **4**(9), 1533–1547 (2013), doi:10.1364/BOE.4.001533.
34. P. Patheja, R. Dasgupta, A. Dube, S. Ahlawat, R. S. Verma, and P. K. Gupta, "The use of optical trap and microbeam to investigate the mechanical and transport characteristics of tunneling nanotubes in tumor spheroids," J. Biophotonics **8**(9), 694–704 (2015).
35. M. Villangca, D. Casey, and J. Glückstad, "Optically-controlled platforms for transfection and single- and sub-cellular surgery," Biophys. Rev. **7**(4), 379–390 (2015).
36. L. Gu and S. K. Mohanty, "Targeted microinjection into cells and retina using optoporation," J. Biomed. Opt. **16**(12), 128003 (2011).
37. S. Batabyal, Y. T. Kim, and S. Mohanty, "Ultrafast laser-assisted spatially targeted optoporation into cortical axons and retinal cells in the eye," J. Biomed. Opt. **22**(6), 60504 (2017).

Biomedical Optics EXPRESS

38. A. M. Wilson, J. Mazzaferri, É. Bergeron, S. Patskovsky, P. Marcoux-Valiquette, S. Costantino, P. Sapieha, and M. Meunier, "In vivo laser-mediated retinal ganglion cell optoporation using KV1.1 conjugated gold nanoparticles," Nano Lett. **18**(11), 6981–6988 (2018).
39. E. K. Shannon, A. Stevens, W. Edrington, Y. Zhao, A. K. Jayasinghe, A. Page-McCaw, and M. S. Hutson, "Multiple mechanisms drive calcium signal dynamics around laser-induced epithelial wounds," Biophys. J. **113**(7), 1623–1635 (2017).
40. K. Schütze, Y. Niyaz, M. Stich, and A. Buchstaller, "Noncontact laser microdissection and catapulting for pure sample capture," Methods Cell Biol. **82**, 649–673 (2007).
41. A. Vogel, V. Horneffer, K. Lorenz, N. Linz, G. Hüttmann, and A. Gebert, "Principles of laser microdissection and catapulting of histologic specimens and live cells," Methods Cell Biol. **82**, 153–205 (2007).
42. J. Fang and B. Schneider, "Laser microdissection: a sample preparation technique for plant micrometabolic profiling," Phytochem. Anal. **25**(4), 307–313 (2014).
43. J. Yamamoto and T. Iwai, "Highly controllable optical tweezers using dynamic electronic holograms," Curr. Pharm. Biotechnol. **13**(14), 2655–2662 (2012).
44. C. Lutz, T. S. Otis, V. DeSars, S. Charpak, D. A. DiGregorio, and V. Emiliani, "Holographic photolysis of caged neurotransmitters," Nat. Methods **5**(9), 821–827 (2008).